PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P1392PC00	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2005/000694	International filing date *(day/month/year)* 21.01.2005	Priority date *(day/month/year)* 21.01.2004
International Patent Classification (IPC) or national classification and IPC INV. C12N7/04 A61K39/12 C12N15/62 C07K14/08 C07K19/00 C12N15/87 C12N15/86 C12N5/10		
Applicant CONSEJO SUPERIOR DE INVESTIGACIONES CIENT... et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

- ☒ Box No. I Basis of the report
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand 21.11.2005	Date of completion of this report 19.06.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Paresce, D Telephone No. +49 89 2399-8995

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2005/000694

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:

   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-24 as originally filed

Sequence listings part of the description, Pages

1-17 as originally filed

Claims, Numbers

1-19 received on 30.12.2005 with letter of 21.12.2005

Drawings, Sheets

1/4-4/4 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2005/000694

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-19
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-19
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: HU Y ET AL: "Chimeric infectious bursal disease virus-like particles expressed in insect cells and purified by immobilized metal affinity chromatography" BIOTECHNOLOGY AND BIOENGINEERING. INCLUDING: SYMPOSIUM BIOTECHNOLOGY IN ENERGY PRODUCTION AND CONSERVATION, JOHN WILEY & SONS. NEW YORK, US, vol. 63, no. 6, 20 June 1999 (1999-06-20), pages 721-729, XP002190336 ISSN: 0006-3592

D2: WO 01/97839 A (RAHAN MERISTEM; STRAM, YEHUDA; ROGEL, ARIE; EDELBAUM, ORIT; SELA, ILAN) 27 December 2001 (2001-12-27)

D3: FERNÁNDEZ-ARIAS A ET AL: "Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles" JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 79, no. part 5, May 1998 (1998-05), pages 1047-1054, XP002218365 ISSN: 0022-1317 cited in the application

D4: MARTINEZ-TORRECUADRADA J L ET AL: "Different Architectures in the Assembly of Infectious Bursal Disease Virus Capsid Proteins Expressed in Insect Cells" VIROLOGY, ACADEMIC PRESS,ORLANDO, US, vol. 278, no. 2, 20 December 2000 (2000-12-20), pages 322-331, XP004435746 ISSN: 0042-6822 cited in the application

D5: MARTINEZ-TORRECUADRADA J L ET AL: "Structure-dependent efficacy of infectious bursal disease virus (IBDV) recombinant vaccines" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 21, no. 23, 4 July 2003 (2003-07-04), pages 3342-3350, XP004429746 ISSN: 0264-410X

D6: US-A-5 788 970 (VAKHARIA ET AL) 4 August 1998 (1998-08-04) cited in the application

The document D1 (Hu et al.) discloses the production of chimeric virus-like particles (VLP's) of IBDV by co-infection of SF9 insect cells with two different recombinant baculoviruses that express, respectively, the whole polyprotein of IBDV, and a Histidine-tagged version of VP2. The resulting chimeric virus-like particles composed of

VP2, VP2H and VP3 are purified by IMAC (see abstract, p. 721-2). The VLPs of D1 are constituted by VP2, VP2H and VP3 and not pVP2 as in the present application.

D2 discloses transgenic plants and bacteria (E. coli) comprising empty particles of the Infectious Bursal Disease Virus (IBDV) which are used as a vaccine for immunizing an avian for protection against IBDV. In one embodiment, the empty particles of the IBDV comprise one or more of virion protein 2 (VP2), virion protein 3 (VP3), and/or virion protein 4 (VP4) (see claims and p. 5-6).

D3 discloses the expression the IBDV VP3, VP2 and VP4 proteins in cells infected with a recombinant vaccinia virus expressing the IBDV polyprotein.

D4 discloses IBDV capsids obtained by expressing the complete IBDV polyprotein in two different baculovirus expression vectors. The VLPs of D4 comprise VP4, VP3 and pVP2.

D5 describes the immunogenicity of VP2, pVP2 and PP particles obtained from cells infected with recombinant baculoviruses encoding IBDV PP, pVP2 and VP2 proteins. The VLPs of D5 comprise VP4, VP3 and pVP2.

D6 discloses a chimeric polypeptide immunogen comprising the VP2 amino acid sequence from a infectious bursal disease virus (IBDV) strain and an epitopic determinant from a 2nd IBDV strain. Virus-Like Particles or three-dimensional particles of natural or recombinant amino acid sequences mimicking the three-dimensional structure of IBDV (encoded by the large genome segment A) but lacking viral RNA are also constructed. Virus-like particles exhibit conformational epitopes exhibited by native viruses of similar sequence. Virus-like particles are created by the proper expression of DNA encoding VP2, VP4, VP3 structural proteins in a proper ORF.

The subject-matter of claims 1-19 has not been made available to the public by any of the available prior art documents and can therefore be regarded as novel (Article 33(2) PCT).

The subject-matter of claims 1-19 cannot be derived from the available prior art in an obvious manner and therefore complies with the requirements of Article 33(3) PCT.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.

PCT/EP2005/000694

CLAIMS

1. An empty capsid of the infectious bursal disease virus (IBDV), VLP(-VP4), characterized in that it is constituted by assembly of only IBDV pVP2 proteins and IBDV VP3 proteins.

2. A nucleic acid characterized in that its nucleotide sequence is constituted by (i) a nucleotide sequence consisting of the open reading frame corresponding to the IBDV pVP2 protein and (ii) a nucleotide sequence consisting of the open reading frame corresponding to the IBDV VP3 protein.

3. A gene construct comprising a nucleic acid according to claim 2.

4. An expression system selected from:

a) an expression system consisting of (i) a gene construct consisting of the open reading frame corresponding to the IBDV pVP2 protein, operatively bound to transcription, and optionally translation, control elements, and (ii) a gene construct consisting of the open reading frame corresponding to the IBDV VP3 protein, operatively bound to transcription, and optionally translation, control elements; and

b) an expression system consisting of a gene construct according to claim 3, operatively bound to transcription, and optionally translation, control elements.

5. An expression system according to claim 4, said expression system being selected from plasmids, bacmids, yeast artificial chromosomes (YACs), bacteria artificial chromosomes (BACs), bacteriophage P1-based artificial chromosomes (PACs), cosmids, and viruses, which, optionally, contain a heterologous replication origin.

6. A host cell containing a nucleic acid according to claim 2, or a gene construct according to claim 3, or an expression system according to anyone of claims 4 or 5.

7. A host cell that is transformed, transfected or infected with an expression system according to anyone of claims 4 or 5.

8. Host cell according to anyone of claims 6 or 7, said cell being an insect cell or a yeast.

9. A process for the production of empty capsids of the infectious bursal disease virus (IBDV), VLPs(-VP4), according to claim 1, comprising culturing a host cell according to anyone of claims 6 to 8, and if so desired, recovering said empty IBDV capsids.

10. Process according to claim 9, wherein said host cell is an insect cell, comprising the steps of:

a) preparing an expression system selected from:

- an expression system constituted by a recombinant baculovirus containing a gene construct according to claim 3, operatively bound to transcription, and optionally translation, control elements; and

- an expression system constituted by (i) a recombinant baculovirus containing a gene construct comprising the open reading frame corresponding to the IBDV pVP2 protein, and (ii) a recombinant baculovirus containing a gene construct comprising the open reading frame corresponding to the IBDV VP3 protein;

b) infecting insect cells with said expression system prepared in step a);

c) culturing the infected insect cells obtained in step b) under conditions allowing the expression of recombinant proteins and their assembly for forming empty IBDV capsids, VLPs(-VP4); and

d) if so desired, isolating and optionally purifying said IBDV empty capsids, VLPs(-VP4).

11. Process according to claim 9, wherein said host cell is a yeast, comprising the steps of:

a) preparing an expression system constituted by a plasmid containing a gene construct according to claim 3;

b) transforming yeast cells with said expression system prepared in step a);

c) culturing the transformed yeasts obtained in step b) under conditions allowing the expression of recombinant proteins and their assembly to form empty IBDV capsids, VLPs(-VP4); and

d) if so desired, isolating and optionally purifying the empty IBDV capsids, VLPs(-VP4).

12. The use of a gene expression system according to anyone of claims 4 or 5 for producing and obtaining empty IBDV capsids, VLPs(-VP4), according to claim 1.

13. The use of empty capsids of the infectious bursal disease virus (IBDV), VLPs(-VP4), according to claim 1 in the manufacture of a medicament.

14. Use according to claim 13, wherein said medicament is a vaccine against the avian disease called infectious bursal disease.

15. Use according to claim 13, wherein said medicament is a gene therapy vector.

16. A vaccine comprising a therapeutically effective amount of empty IBDV capsids, VLPs(-VP4), according to claim 1, optionally together with one or more pharmaceutically acceptable adjuvants and/or vehicles.

17. Vaccine according to claim 16 to protect birds from the infection caused by the infectious bursal disease virus (IBDV).

18. Vaccine according to claim 17, wherein said birds are selected from the group formed by chickens, turkeys, geese, ganders, pheasants, quails and ostriches.

19. Vaccine to protect chickens from the infection caused by the infectious bursal disease virus (IBDV) comprising a therapeutically effective amount of empty IBDV capsids, VLPs(-VP4), according to claim 1, optionally together with one or more pharmaceutically acceptable adjuvants and/or vehicles.